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Dose Response Effects of Dermally applied Diethanolamine on Neurogenesis in Fetal Mouse Hippocampus and Potential Exposure of Humans

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Diethanolamine (DEA) is a common ingredient of personal care products. Dermal administration of DEA diminishes hepatic stores of the essential nutrient choline and alters brain development. We previously reported that 80 mg/kg/day of DEA during pregnancy in mice reduced neurogenesis and increased apoptosis in the fetal hippocampus. This study was designed to establish the dose-response relationships for this effect of DEA. Timed-pregnant C57BL/6 mouse dams were dosed dermally from gestation day 7–17 with DEA at 0 (controls), 5, 40, 60, and 80 mg/kg body/day. Fetuses (embryonic day 17 [E17]) from dams treated dermally with 80 mg/kg body/day DEA had decreased neural progenitor cell mitosis at the ventricular surface of the ventricular zone (hippocampus, 54.1 ± 5.5%; cortex, 58.9 ± 6.8%; compared to controls; $p < 0.01$). Also, this dose of DEA to dams increased rates of apoptosis in E17 fetal hippocampus (to 177.2 ± 21.5% of control; measured using activated caspase-3; $p < 0.01$). This dose of DEA resulted in accumulation of DEA and its metabolites in liver and in plasma. At doses of DEA less than 80 mg/kg body/day to dams, there were no differences between treated and control groups. In a small group of human subjects, dermal treatment for 1 month with a commercially available skin lotion containing 1.8 mg DEA per gram resulted in detectable plasma concentrations of DEA and dimethyldiethanolamine, but these were far below those concentrations associated with perturbed brain development in the mouse.

Key Words: **diethanolamine; choline; pregnancy; brain development; mouse; hippocampus.**

Diethanolamine (DEA) (Chemical Abstracts Registration Number 111-42-2) is widely used as a chemical intermediate, as an anticorrosion agent in metalworking fluids and as a surfactant component in cosmetic formulations, pharmaceuticals, and agricultural products (CIR, 1983, 1986). The most probable route of environmental exposure to DEA in humans is via dermal exposure to personal care products (i.e., skin lotions, soaps, shampoos, and cosmetics), detergents, and other surfactants that contain DEA; cosmetic formulations may have concentrations of DEA ranging from 0.1 to 5% (CIR, 1983).

DEA is structurally similar to the essential nutrient choline, and DEA treatment in rodents perturbed choline metabolite concentrations in liver (Lehman-McKeeman *et al*., 2002; Stott *et al*., 2000). DEA is metabolized by routes common to endogenous alkanolamines (ethanolamine and choline) and is incorporated into phospholipids in liver, kidney, spleen, and brain of mice and rats (Mathews *et al*., 1995), probably by the enzyme-catalyzed transphosphatidylation of phosphatidylcholine (PtdCho) by phospholipase D (Dippe *et al*., 2008). Furthermore, DEA inhibits PtdCho synthesis in rat liver tissue *in vitro* (Barbee and Hartung, 1979) and *in vivo* (Browning and Snyder, 1987). Also, DEA is an inhibitor of choline transport into neural progenitor cells (Niculescu *et al*., 2007).

Maternal choline deficiency during pregnancy in the rodent results in diminished proliferation and increased apoptosis of neural progenitor cells in the fetal hippocampus (Albright *et al*., 1999; Craciunescu *et al*., 2003), and these result in lifelong changes in memory performance (Meck and Williams, 2003; Meck *et al*., 1988). Similarly, DEA administration (80 mg/kg/day) during pregnancy caused diminished proliferation and increased apoptosis of neural progenitor cells in the fetal hippocampus (Craciunescu *et al*., 2006), probably by perturbing choline metabolism. The present study reports that the effects of DEA during pregnancy were only observed at the highest dose that was tested (80 mg/kg body weight/day to the dams from days 11 to 17 or pregnancy) and were not detected at lower doses. These exposure data should be useful in the evaluation of the safety of cosmetic and other personal care products and ingredients containing DEA.

MATERIALS AND METHODS

Animals

Timed-pregnant C57BL/6 mice (the morning when the vaginal plug was observed after mating was considered embryonic day 0 of gestation) were used

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in all experiments and were obtained from The Jackson Laboratories (Bar Harbor, ME) on day 7 of gestation, maintained in a climate-controlled environment and exposed to a 12-h light/dark cycle daily. All animal protocols were approved by the UNC Institutional Animal Use Committee. Mice were fed purified AIN-76A diet containing 1.1 g/kg (7.8 mmol/kg) choline chloride (Dyets, Bethlehem, PA) and water *ad libitum* unless otherwise noted.

A total of 59 female mice were used; three groups of seven mice were treated with vehicle (control) from gestation day embryonic day 7 (E7) through E17, one group of seven mice for each DEA dose (5, 40, 60, and 80 mg/kg body weight) was treated for the same period, one group of five nonpregnant female mice was treated with vehicle for 11 days, and one group of five nonpregnant female mice was treated with 80 mg/kg body weight/day DEA for 11 days. The animals were dosed dermally with DEA (BioUltra > 99.5% purity; Sigma-Aldrich, St Louis, MO) dissolved in acetone at 0, 5, 40, 60, and 80 mg/kg body weight/day from gestation day E7 through E17. The dose formulations were prepared so as to deliver the desired dose in the same volume of acetone (1.78 μl/g mouse weight) over the prepared target site using a micropipetter equipped with a blunted tip. The dose formulations were stored at 4°C, protected from light, in polyethylene vials. All dose formulation samples were within 5% of the target concentrations (we analyzed the composition of the last vial used in each treatment using the liquid chromatography-electrospray ionization-mass spectrometry [LC-ESI-MS] method described later). Dermal application of DEA to pregnant dams and tissue collection on gestational day 17 were conducted as described previously (Craciunescu *et al*., 2006).

Two male fetal brains from each dam were embedded in paraffin, and 5-μm coronal serial sections containing the brain regions of interest such as the hippocampus and cortex were cut and applied on glass slides for histological and immunohistochemical assays. Since there is a posterior to anterior gradient of neurogenesis in fetal mouse brain, the sections were reviewed at the time of immunostaining to ensure that they included anatomically reproducible areas of the hippocampus as defined by a standard atlas of the developing brain (Jacobowitz and Abbott, 1998). In prenatal and early postnatal stages, neurogenesis occurs mostly within two relatively thin layers of tissue lining the primitive ventricular cavities, referred to as the ventricular zone (VZ) and the subventricular zone (SVZ) (The Boulder Committee 1970)

Each immunohistochemical assay was replicated on subsequent serial sections and on littermate male fetus brains.

Human Subjects

Healthy premenopausal female subjects (aged 20–45 years) of any race or ethnicity were recruited from the local area for a 1-month study approved by the Public Health Institutional Review Board at the University of North Carolina at Chapel Hill. The study was advertised using IRB-approved flyers placed on bulletin boards in target locations. Prospective subjects who contacted us with an interest in the study were prescreened by telephone. Inclusion was contingent on a good state of health (no acute or chronic disease) and willingness to participate in an outpatient setting. Women who were pregnant or planning to get pregnant in the next 6 months were excluded. Three prospective subjects indicated that they had an interest in the study; all three met eligibility criteria and were enrolled. The three subjects were 30, 30, and 29 years of age, two were Caucasian, and one was Asian. Informed consent, approved by the UNC Public Health IRB, was obtained from all subjects prior to initiating any study-related procedure.

Subjects were asked to come in for three study visits—day 0 (baseline), 1 week, and 1 month (end of treatment). At the baseline visit, subjects were given a supply of 929 g of a commercially available body lotion (containing 1.8 mg DEA/g lotion measured using the LC-ESI-MS method described later) placed in an unmarked bottle. Subjects were blinded against the product's brand identification. Subjects were asked to begin using the lotion the following day (day 1) and to continue applying the lotion for the duration of the study (1 month). The subjects were instructed to apply the lotion to their whole body (per the manufacturer's instructions) twice per day—in the morning after showering and again before bedtime. Compliance with the body lotion regimen was monitored by having the participants keep a log of daily application and by visually inspecting and weighing the bottle of lotion at the 1-week and 1-month follow-up visits. At each visit, subjects were monitored for pregnancy using a commercially available urine pregnancy test. Whole blood (7 ml) was collected in EDTA BD Vacutainer tubes by venipuncture at the baseline (1 day before treatment), 1-week, and 1-month (end of treatment) study visits, between 8:00 and 10:00 A.M. DEA and metabolites were assayed in plasma. Two subjects completed the study per protocol and used the body lotion for the full 1-month period. One subject voluntarily dropped out of the study after 3 weeks of participation. For this subject, a 3-week whole-blood sample was collected in place of the 1-month measurement.

Assessment of Mitosis

The mitotic and synthetic zone of the VZ adjacent to the lateral ventricle is the region of fetal hippocampus from which neuronal and glial type cells originate (Altman and Bayer, 1990). The cells at the ventricular surface of the VZ of the developing hippocampus include progenitor cells that have exited the S phase and entered the mitotic phase of the cell cycle. To determine whether mitosis in fetal hippocampus was altered by maternal dermally administered DEA during pregnancy, coronal sections were probed with a rabbit polyclonal antibody that recognizes phosphorylated histone H3 (phospho-H3; Upstate, Lake Placid, NY) as previously described (Craciunescu *et al*., 2006). Histone H3 is the core protein of the nucleosome that becomes Ser-10 phosphorylated in the G_2/M phase (Hendzel *et al*., 1997). This phosphorylation is essential for the maintenance of mitosis-associated chromosome condensation (Hans and Dimitrov, 2001).

The incidence of phospho-histone H3-labeled cells was measured at the ventricular surface of the VZ beginning at the junction of the hippocampus and choroid plexus (hippocampal wedge [Altman and Bayer, 1990]) and extending toward and over the cortical VZ. Cells were counted at a final magnification of ×200 in 6–12 hippocampal hemispheres from three to six consecutive serial sections, and the values were averaged to obtain a single value/hippocampus (cortex)/animal. Calibrated ×40 magnification images of the same regions were used to measure the length of the hippocampal SVZ with an internal macro of NIH Image J program version 1.61. Analyses were replicated on subsequent serial sections, with similar results.

Assessment of Apoptosis

Activated caspase-3 immunoreactivity was used to detect apoptotic cells in fetal hippocampus. The pretreatment of slides for deparaffinization, antigen retrieval, and blocking was identical to the one for mitosis assessment and was followed by overnight incubation with a primary antibody to cleaved (activated) caspase-3 (Asp175) (Cell Signaling Technology, Beverly, MA). Then, goat Cy3-anti-rabbit IgG (Chemicon, Temecula, CA) was applied for 2 h at room temperature to display the activated caspase-3. 4',6-Diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St Louis, MO), 0.1 μg/ml, for 20 min was used to counterstain nuclear DNA. The activated caspase-3-positive cells were identified and scored by a trained observer, blinded to the animal's grouping, based upon the presence of yellow fluorescent staining for activated caspase-3. Stained nuclei were usually, but not always, condensed, and intense blue fluorescent chromatin was often visible inside, consistent with fragmented DNA. Apoptotic indices for the fetal mouse brain hippocampus of the different animal groupings are presented as the number of apoptotic cells/hippocampal section. In all, 6–12 hippocampal hemispheres from three to six consecutive serial sections were averaged to obtain single value/hippocampal section/animal.

Image Analysis

For mitosis assessment and activated caspase-3 evaluation, the image analysis of fetal brain slices was performed using a Zeiss Confocal Laser Scanning Microscope LSM 210 (Carl Zeiss, Thornwood, NY) equipped with an Optronics DEI 750 low light level–integrating CCD camera (Optronics Engineering, Goleta, CA) connected to an Apple Macintosh G3 computer utilizing a Scion CG7 image capture card for digital image capture of standard and epifluorescence images and the public domain NIH Image J program version 1.61. For the replicated experiments, the apoptotic cells were detected

hemilaterally in each of the selected sections after labeling for activated caspase-3 using a Nikon FXA microscope (Nikon, Garden City, NY) equipped with an Optronics TEC-470 CCD Video Camera System (Optronics Engineering) and the public domain NIH Image J program version 1.61. All images were captured by using ×5, ×20, or ×40 objectives and fluorescent filters optimized for observing DAPI (blue), erythrocyte autofluorescence (green, using filter XF 100-2; Omega Opticals, Brattleboro, VT), and Cy3 conjugates (yellow, XF 108-2; Omega Opticals) signals, respectively. Images obtained from the same field with different fluorescent probes were subsequently overlapped or merged.

Analysis of Choline and Diethanolamine Compounds

Reagents. Betaine, choline chloride, glycerophosphocholine (GPCho), phosphocholine (PCho), DEA, and N-methyldiethanolamine were ordered from Sigma Chemicals (St Louis, MO). Bis(2-hydroxyethyl)dimethylammonium chloride was obtained from Acros Organic (Fair Lawn, NJ). Choline-[N,N,N-trimethyl-d9] bromide, betaine-[N,N,N-trimethyl-d9] hydrochloride, and phosphocholine-[N,N,N-trimethyl-d9] chloride were purchased from CNS isotopes (Quebec, Canada). Glycerophosphocholine-[N,N,N-trimethyl-d9] was synthesized as described elsewhere (Koc *et al*., 2002). 1,2-Dipalmitoyl-sn-3-glycerophosphocholine-[N,N,N-trimethyl-d9] was purchased from Avanti Polar Lipids (Alabaster, AL). Bis(2-hydroxyethyl)-d8-amine was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). All solvents used were of high-performance liquid chromatography grade.

LC-ESI-MS analysis. Choline, betaine, PCho, GPCho, PtdCho, and sphingomyelin (SM) were determined in maternal liver using liquid chromatography-electrospray ionization-isotope dilution mass spectrometry (LC-ESI-IDMS; LCQ quadrupole ion trap mass spectrometer equipped with an API2 electrospray ionization source; ThermoQuest, San Jose, CA) as previously described (Koc *et al*., 2002). The same method, using selected reaction monitoring mode, was used for analysis of DEA and its metabolites in tissues, dose formulations, and body lotion. For DEA (*m/z* 106), monomethyldiethanolamine (MDEA) (*m/z* 120) and dimethyldiethanolamine (DMDEA) (*m/z* 134) in aqueous phase, *m/z* value of 88, 102, and 90, respectively, were monitored with DEA-d8 (*m/z* 96) as an internal standard. For phosphatidyldiethanolamine (PtdDEA), phosphatidylmonomethyldiethanolamine (PtdMDEA), and phosphatidyldimethyldiethanolamine (PtdDMDEA), a source collision energy of 35 eV, followed by and a further collision energy of 25 eV was used to achieve the characterization fragment (*m/z* 88, 102, 116, and 104) since no commercial standards were available (PtdCho-d9 was used as an internal standard).

Briefly, deuterium-labeled internal standards of choline, betaine, DEA, GPCho, PCho, SM, and PtdCho and 400 μl of methanol/chloroform (2:1, vol/vol) were added to 100-mg aliquots of tissue. Samples were vortexed vigorously and left at – 20°C overnight. At the end of the extraction, samples were subjected to centrifugation at 1500 × g for 5 min at room temperature. The supernatant was transferred to a new tube, and the residue was reextracted with 250 μl of methanol/chloroform/water (2:1:0.8, by volume). The supernatants from both extractions were combined. To the combined solution, 100 μl of chloroform and then 100 μl of water were added to form two phases. After centrifugation at 1500 × g for 5 min, the aqueous phase (which contained choline) was separated from the chloroform phase (which contained PtdCho and SM). A 10-μl aliquot of the lower organic phase was analyzed by LC-ESI-IDMS directly. The aqueous phase was dried by vacuum centrifugation (SpeedVac; Savant Instruments, Farmingdale, NY) and redissolved in 20 μl of water. Following addition of 200 μl of methanol, the aqueous phase was subjected to centrifugation at 1500 × g for 5 min to remove the precipitated unknown compounds from the solution. A 10-μl aliquot of this solution was then analyzed by LC-ESI-IDMS.

Data collection and analysis were performed by Xcalibur software (ThermoQuest, San Jose, CA) running on Windows NT4.0.

Statistical Analysis

All statistical analyses were performed with JMP software (V 2; SAS Institute, Cary, NC). Data are presented as mean ± SE.

RESULTS

DEA and Choline Metabolites after Treatment

Treatment of adult female mice with 80 mg/kg body/day DEA for 11 days increased concentrations of DEA, MDEA, DMDEA, PtdDEA, PtdMDEA, and PtdDMDEA in liver and plasma (Table 1). Many of the DEA metabolites achieved higher concentrations in liver than in plasma (Table 1). Application of commercial body lotion containing DEA to the skin of humans for 1 month also resulted in increased concentrations of DEA and DMDEA in plasma (Fig. 1), though the concentrations achieved were approximately 100- to 200-fold lower than those observed in mice after the 80 mg/kg body/day DEA dose for 11 days. Blanks, run simultaneously, were at least twofold lower than the values measured in plasma or liver from untreated control mice or humans.

Treatment of adult female mice with 80 mg/kg body/day DEA for 11 days decreased hepatic concentrations of choline and all its metabolites (Table 2). PCho, a major storage pool of choline in liver, was decreased to 38% of control ($p < 0.01$); PtdCho, another major storage pool, was decreased to 53% of control ($p < 0.01$); and betaine, a metabolite of choline used as a methyl donor, was decreased to 41% of control ($p < 0.01$).

Mitosis in Fetal Brain

Proliferation of neural progenitor cells in E17 brain VZs of hippocampus and cortex was assessed using immunohistochemistry for phospho-histone H3. Typical distribution of mitotic cells in periventricular zones is presented in Figure 2, upper panels. In both regions studied, only the treatment with 80 mg/kg/day DEA diminished the proportion of cells that were in the mitotic phase to approximately 50% of control ($p <$ 0.01 different from control; Fig. 3; for cortical VZ, the data are

TABLE 1
DEA and Its Methylated Metabolites Accumulated in Liver and Plasma of Female Mice Dermally Treated with 80 mg DEA/kg body/day

	Liver (nmol/g)		Plasma (nmol/ml)	
	Control	DEA	Control	DEA
DEA	0	8958 ± 965**	58 ± 27	1253 ± 127**
MDEA	100 ± 13	1549 ± 219**	0	405 ± 76**
DMDEA	3.2 ± 0.6	1216 ± 101**	14 ± 6	106 ± 16**
PtdDEA	0	2.4 ± 0.3**	0	4.3 ± 1.1**
PtdMDEA	0	24 ± 2**	0	11 ± 2**
PtdDMDEA	0	137 ± 8**	0	243 ± 17**

Note. Female mice were dermally treated with 80 mg/kg body/day DEA or vehicle control for 11 days as described in the text. DEA and its metabolites were assayed in plasma and liver using LC-ESI-IDMS. Results are presented as mean ± SE; $n = 5$ animals/group. **$p < 0.01$ DEA-treated group differs from the control group by ANOVA and Tukey-Kramer test.

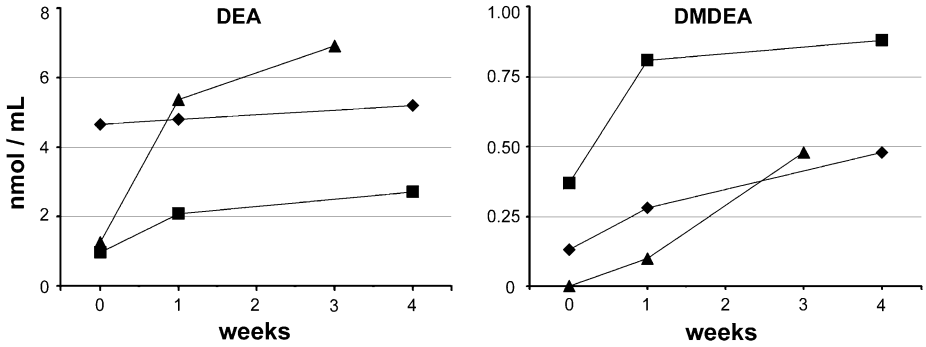

FIG. 1. DEA and its metabolite DMDEA accumulated in humans who applied DEA-containing lotion to their skin. Three healthy women applied a commercially available skin lotion that contained DEA (0.18% wt/vol) each day for the time indicated as described in the text. DEA and its metabolites were assayed in plasma using LC-ESI-IDMS. Data from each subject are separately presented.

similar to that from hippocampus and are not shown). DEA treatment did not alter the length of the VZs.

Apoptosis in Fetal Brain

Apoptosis was assessed in E17 brain hippocampus using immunohistochemistry for activated caspase-3. Typical apoptotic cells expressing activated caspase-3 in hippocampus are shown in Figure 2, lower panels. The number of apoptotic cells was increased 1.75-fold in fetuses from 80 mg/kg body/day DEA-treated dams compared to the controls ($p < 0.01$, Fig. 4). Apoptosis was not increased at any of the lower doses of DEA.

DISCUSSION

The present study confirmed earlier results that showed that 80 mg/kg/day DEA administered to pregnant mouse dams resulted in depletion of choline metabolites in maternal liver as well as in diminished neural progenitor cell proliferation and increased apoptosis in fetal brain (Craciunescu *et al.*, 2006). Also confirmed was the observation that hepatic stores of choline were depleted in nonpregnant rats treated with DEA (Lehman-McKeeman *et al.*, 2002). The present study now adds the observations that DEA treatment resulted in accumulation of DEA and its metabolites in maternal liver and plasma, that humans applying commercial skin lotion also absorb small amounts of DEA, and that the lowest effect (altered fetal brain progenitor cell proliferation or apoptosis) level of DEA in mice is at or near to 80 mg/kg/day applied dermally.

DEA and its N-methylated metabolites accumulated in both liver and plasma of treated mice (Table 1) achieving 10-fold higher concentrations in tissues than in blood. We confirmed earlier observations (Mathews *et al.*, 1995) that DEA can substitute for the choline head group in membrane phospholipids, forming PtdDEA, PtdMDEA, and PtdDMDEA. This could alter membrane function. Untreated mice had detectable (well above blank), though low, concentrations of DEA metabolites in liver and plasma. This suggests that there is an environmental source of exposure in laboratory mice, perhaps, from solutions used to wash cages or bottles.

Human exposure to alkanolamines such as DEA occurs mainly through the skin (Henriks-Eckerman *et al.*, 2007). Four weeks of application of a commercially available DEA-containing body lotion to a small group of humans resulted in plasma concentrations of DEA and metabolites that were significantly increased above blank values but that were approximately 100- to 200-fold lower than those achieved in mice treated with the 80 mg/kg/day dose. The subjects applied between 14.5 and 22.9 ml/day (based on returned bottle content of lotion). A 60-kg person applying 20 ml/day of this strength lotion was exposed to a DEA dose of 0.6 mg/kg/day. In a few weeks of application, the three subjects achieved concentrations of DEA and metabolites in plasma that were approximately 0.5–1% of the concentrations achieved after the mice were treated with 80 mg/kg/day for 11 days. Since the

TABLE 2
Concentrations of Choline and Its Metabolites Decreased in Liver of Female Mice Dermally Treated with 80 mg DEA/kg body/day

	Liver (nmol/g)	
	Control	DEA
Choline	777 ± 71	232 ± 14**
PtdCho	15,407 ± 1464	8192 ± 527**
SM	1351 ± 52	906 ± 35*
Betaine	543 ± 73	223 ± 68**
GPCho	157 ± 55	9.5 ± 3.6**
PCho	300 ± 24	113 ± 8**

Note. Female mice were dermally treated with 80 mg/kg body/day DEA or vehicle control for 11 days as described in text. Choline and its metabolites were assayed using LC-ESI-IDMS. Results are presented as the mean ± SE; n=5 animals/group. *$p < 0.05$; **$p < 0.01$ DEA-treated group differs from the control group by ANOVA and Tukey-Kramer test.

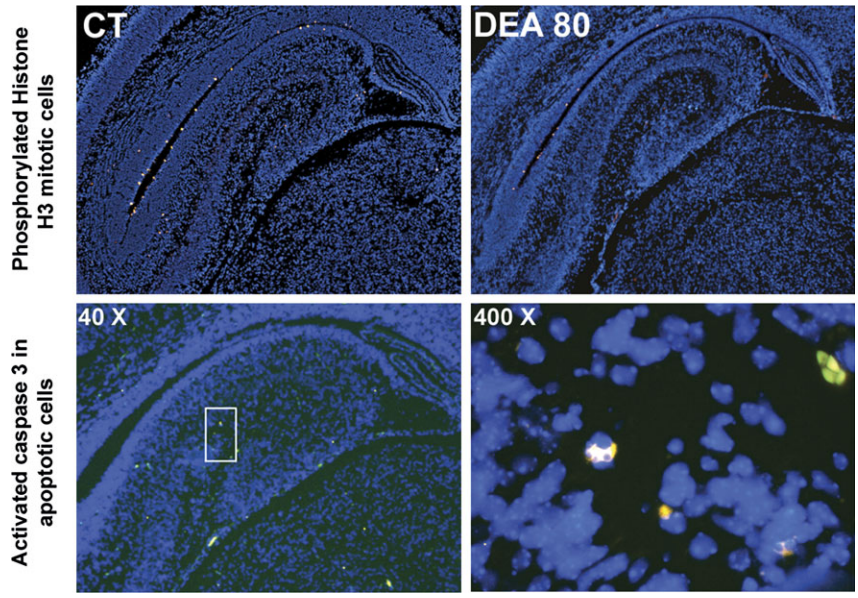

FIG. 2. Immunohistochemical analysis of cell proliferation and caspase-3 activation in fetal E17 mouse hippocampus. Pregnant mice were treated dermally with 80 mg/kg/day DEA (or vehicle control) from E7 until they were killed on E17. Coronal sections were prepared from the brains of fetuses from each group for the analysis of mitosis using the mitosis-specific marker phosphorylated histone H3 and apoptosis as described in text. Representative images for mitosis in fetal brain hippocampus at ×40 magnification are shown in the upper panels; CT for controls (results were similar for all the no-effect doses; 5, 40, and 60 mg/kg/day DEA). The boxed area in the lower left panel is shown at ×400 magnification in the lower right panel and presents active caspase-3-positive cells. The DAPI nuclear DNA counterstaining is blue, while the Cy3 conjugated secondary antibody bound to the anti-phospho-histone H3 (ser10) or anti-active caspase-3 primary antibody stains bright yellow.

dose administered was 133-fold lower, it is not surprising that the concentrations achieved were proportionately lower. It is noticeable that all three subjects had low, but detectable, concentrations of DEA in plasma at baseline (Fig. 1), suggesting that humans are exposed to DEA in the environment. The human data from the present study should be considered as pilot data; only three subjects were studied (one for 3 weeks rather than a month), and there was significant variability among subjects. A larger group should be studied prior to making definitive conclusions and quantitative risk estimates using this data. The human data are presented to provide an estimate of the order or magnitude of response to exposure in humans relative to the mouse.

Because personal care products and cosmetics (CIR, 1983) contain no more than 5% DEA and human skin is not very permeable to DEA (Brain *et al*., 2005; Kraeling *et al*., 2004), it is unlikely that humans using these products will be exposed to the amounts of DEA that we report to have effects on mouse brain development (based on the current study, if a 60-kg body weight human used 20 ml of a product containing 5% DEA daily, estimated exposure would be 16.6 mg/kg/day.) Occupational exposure to DEA is most likely through the use of lubricating liquids in machining operations; DEA is a component of bulk cutting fluids, which contain 4–5% DEA by weight but are normally diluted 20-fold before use (Technology Planning and Management Corporation, 2002). The National Institute for Occupational Safety and Health estimates that the number of workers potentially exposed to DEA is approximately 800,000/year (Technology Planning and Management Corporation, 2002). Annual production of DEA in the United States was estimated at 106,000 tons in 1995

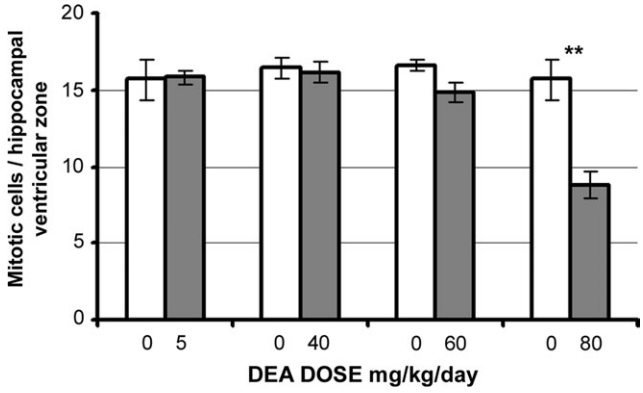

FIG. 3. Quantitative analysis of mitosis in hippocampal sections from fetal mice at E17. Pregnant mice were treated with DEA and fetal hippocampal sections and prepared as presented in Figure 2. Images were analyzed in an unbiased blinded manner as described in text. Pooled data from the dose treatment experiments are presented as mean number of mitotic cells (±SE) at the ventricular surface of the hippocampal VZ. Open bars = vehicle control; shaded bars = DEA dose treated. Similar results (data not shown) were obtained for the ventricular surface of cortical VZ. Results are presented as the mean ± SE. $n = 7$ animals per group; $^{**}p < 0.01$ different from control in same brain region by ANOVA and Tukey-Kramer test.

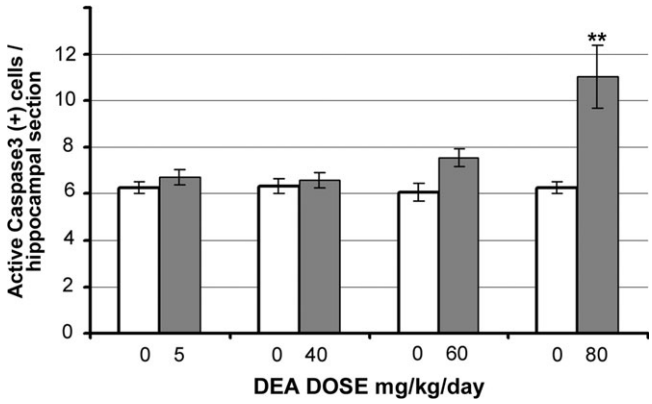

FIG. 4. Maternal DEA treatment increases apoptosis in fetal mouse hippocampus on E17. Pregnant mice were treated with different doses of DEA, and fetal hippocampal sections were assessed as presented in Figure 1. These were analyzed using activated cleaved caspase-3 immunoreactivity as a measure of apoptosis as described in text. Open bars = vehicle control; shaded bars = DEA dose treated. Cell counts from 6–12 hippocampal sections are presented as mean number of positive cells ± SE; $n = 7$ animals per group. $^{**}p < 0.01$ different from control by ANOVA and Tukey-Kramer test.

(Technology Planning and Management Corporation, 2002). Again, rough calculations suggest that occupational exposure to DEA will not approach the 80 mg/kg/day dose needed to alter neurogenesis in mice. These conclusions should be used cautiously as it is possible that human embryonic hippocampus is more sensitive to DEA than is mouse hippocampus. Also, the present study only administered DEA lotion to subjects for 1 month; humans could use such products daily for a lifetime. Excellent reviews exist that summarize the toxicology of DEA (IARC, 2000; Leung *et al*., 2005).

This study identifies the dose-response relationship for mouse embryotoxicity of DEA, a common commercial ingredient in personal care goods, and we present pilot data that suggest that human exposure is likely to be on the order of 100- to 200-fold lower than that needed to perturb brain development in the mouse.

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